

## Short Communication

### Phylogenetic Analysis of Peste des Petits Ruminants Virus Isolated from District Gujranwala, Pakistan

Asad Amanat Ali<sup>1\*</sup>, Muhammad Iftikhar<sup>1</sup>, Syed Maaz Nadeem<sup>2</sup>, Atta Subhani<sup>3</sup>, Christine Pohlke<sup>4</sup>

<sup>1</sup>Poultry Research Institute Rawalpindi, Pakistan; <sup>2</sup>Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan; <sup>3</sup>Veterinary Officer, Livestock and Dairy Development Department, Punjab, Pakistan; <sup>4</sup>Laboratory of Molecular Neurobiology, University of Greifswald, Germany,

\*Corresponding author: asadamanatali@gmail.com; Phone: +923334604256

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#### ABSTRACT

Peste des petits ruminants (PPR) disease is an endemic disease in Pakistan and its neighboring countries. The blood samples from sheep and goats suffering from PPR were taken from district Gujranwala Pakistan to investigate the molecular nature and geographical distribution of Peste des petits ruminants virus (PPRV) circulating in Gujranwala. The nucleoprotein (N) was amplified by using RT-PCR, product was further subject to conventional PCR to get multiple copies of it, then sequenced and aligned with the available corresponding sequences and phylogenetic tree was constructed. The results provide strong evidence of presence of expected Asian lineage IV. The PPRV isolates perceived in district Gujranwala showed high degree of homology with the PPRV isolates of Tajikistan. The current study delivered information of topical spread of PPRV lineage IV from neighboring proximity to the small ruminant in district Gujranwala. Thus it is very crucial to characterize the PPRV in the whole country to control the spread of disease.

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Peste des petits ruminants (PPR) is a highly contagious viral disease of domestic and wild cloven footed animals. Peste des petits ruminants virus (PPRV) belongs to *Morbillivirus* in the family *Paramyxoviridae* (Liu et al., 2010). It is differentiated by mouth ulceration, high fever, diarrhea, and pneumonia and also known as stomatitis pneumo-enteritis complex (Munir et al., 2012a). The PPRV was first reported in 1942 by Gargadennec and Lalanne when Rinderpest like symptoms was seen in sheep and goat (Gargadennec & Lalanne, 1942). Due to a very high morbidity (100%) and mortality (90%), this disease has become a major economic threat to livestock population in intertropical regions of the Arabian Peninsula, Africa and Asia (Kwiatk et al., 2010). The PPRV is a nonsegmented negative sense single stranded RNA virus and its genome consists of 15,948 nucleotides (Bailey et al., 2005). This nonsegmented PPRV genome encodes six structural proteins like nucleocapsid (N), phosphoprotein (P), fusion protein (F), matrix protein (M) and hemagglutinin (H) and two non-structural proteins C and V. Moreover M, H and F genes are linked with the host associated envelop. The M protein is derived from the surface and nucleocapsid protein (Majid et al., 2011). The F protein aids in the penetration of virus into the host cell membrane and also plays a critical role in the induction of the host immune response (Majid et al., 2011). The F and N genes are a well conserved set of genes. The mean nucleotides variability is not more than 10% among the most indistinctly related genes and did not exceed 30% in some parts of the sequences (Chard et al., 2008). However the N gene is a more sensitive and copiously transcribed PPRV gene (Barrett et al., 2006).

The genotyping classification and sequence analysis has revealed four lineages of F and N genes which are distinctive geographically and hence provide an efficient tool to survey the

PPRV prevailing worldwide (Ozkulet et al., 2002). The viruses of Lineages I and II are more abundantly isolated and restricted in western and central African countries which are the countries of the origin of PPRV. The viruses of lineage of III are restricted to eastern Africa and some parts of Middle East like Qatar, Yemen and Oman. The virus belongs to lineages III also exclusively isolated from southern parts of India and the lineage IV has only been isolated from Asia (Shaila et al., 1996).

The PPRV has been suggested to circulate mutely and acts as opportunistic pathogen and infected the host population when their immunity level drops and cause sporadic outbreaks. Consistent outbreaks have been reported every year by the World Organization for Animal Health. The outbreaks likely to happened when the immune naive animals have direct contact with the animals of endemic areas. In Pakistan the PPR has been reported since 1991 when the epidemic was experienced in the Punjab Province. These outbreaks that have been reported in different periods of time are based on the clinical observations and epidemiological studies. PPRV was characterized in Pakistan during 1994 through PCR (Amjad et al., 1996). After that another outbreak was reported in 2005 when the goats were found seropositive for PPRV antibodies in the Okara region of Punjab Province (Ahmad et al., 2005). More outbreaks were reported in the northern Pakistan and south and central Punjab (Durrani et al., 2010). The outbreak was also reported in 2008 in district Lahore which is adjacent to district Gujranwala (Rashid et al., 2008). The virus prevalence was confirmed by agar gel immunodiffusion by Rashid et al., 2008. The PPR considered as endemic diseases of sheep and goat in Pakistan especially in the Punjab province where the population of sheep and goat is higher as compared to other provinces. Different vaccination programs were introduced

with the live attenuated Virus belonging to Lineage I. Despite of the strict vaccination programs and other preventive and clinical measures the PPR outbreaks are frequent. The PPRV is a contagious virus and it has the ability of spreading through the direct contact with the nasal secretions and intestinal excretions of the infected animals to the healthy animals in close proximity (Ezeibe et al., 2008). In spite of only one serotype the virulence nature of PPRV differ from strain to strain and due to the involvement of secondary infection clinical diagnosis and differential diagnosis with other diseases like pasteurellosis, caprine pleuropneumonia, coccidiosis, FMD and contagious ecthyma has become complicated (Emikpe et al., 2010). The current study has been designed to determine the molecular classification, genetic diversity and phylogenetic relationship of PPRV circulating in district Gujranwala of Punjab Province in Pakistan.

The blood samples were collected from PPR suspected diseased animals from September to December 2012 from sheep (n=14) and goat (n=18) within the vicinity of district Gujranwala. All the animals were suffering from respiratory disease with high fever ranges 104-106°F. Other signs include mucopurulent nasal discharge, severe bronchopneumonia, necrotic stomatitis, and profuse diarrhea. All animals died after 7 to 13 days of clinical signs. The 200µl of whole blood samples from individual animals was taken and was spotted on QIAcard FTA indicator Four Spots (Qiagen, Hilden, Germany). The QIAcard's function is to lyse host and virus cells and to preserve the genomic

material. The QIAcards has the ease of transportation of blood samples. Total RNA was eluted from QIAcard as described before (Munir et al., 2012b). The viral N gene (Accession No: Q08823) was targeted by employing real-time RT-PCR to confirm the presence of PPRV from blood samples by using Rotor-Gene 6000 real-time analyzer by Qiagen (Kwiatek et al., 2010). AgPath ID one-step RT-PCR kit (Applied Biosystem, USA) was used in this study. FAM was employed as reporter dye, which shows amplification after every annealing step of each cycle. The samples that showed threshold cycle (Ct) values <35 were consider positive for PPRV. A total of 9 samples (sheep, n=4 and goat, n=5) were found positive and then these samples were further amplified in a conventional PCR with hypervariable 3'-end of the N gene using primers NP3 (5'-TCTCGGAAATCGCCTCACAGACTG-3') and NP4 (5'-CCTCCTCTGGTCTCCAGAATCT-3') (Couacy-Hymann et al., 2002). The PCR products were extracted from the gel and cloned in pCR3.0 vector by using TOPO TA cloning kit (Invitrogen, USA). The sequencing of N gene from each outbreak (sheep and goat) was sequenced by using ABI PRISM BigDye Terminator version 3.1 (Applied Biosystems, USA). The homologies of the sequences from both outbreaks were found as 100% in this study. The sequences then were aligned with all published sequences based on gene bank representing all lineages of PPRV. The neighbor-joining method and Kimura two-parameter model in MEGA5 (CEMI, Tempe, AZ, USA) was employed to built a phylogenetic tree.

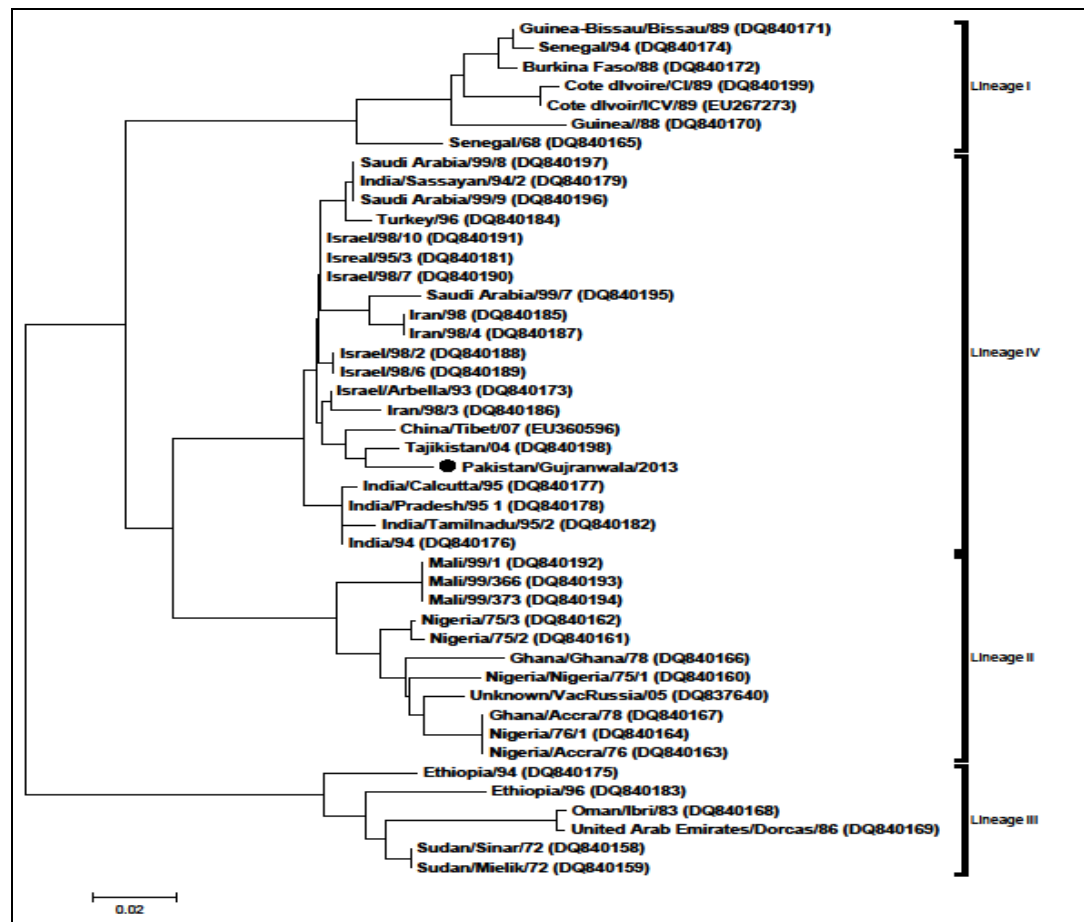


Figure 1. Neighbor-joining consensus tree based on N gene of Peste des petits ruminants virus created with Kimura two-parameter model in MEGA version 5. The 1000 replicates are the bootstrap value and the value above 30% shown. The horizontal distance is directly proportional to sequence difference. The sample which was used in this study marked as (●).

The aim of this study was molecular characterization and

estimation of genetic relationship of PPRV isolates obtained

from district Gujranwala with the other available PPRV sequences. The analysis of the 9 positive samples confirmed the presence of PPRV in Gujranwala. District Gujranwala has central importance in Punjab province in terms of Livestock population. A lot of PPR outbreaks have been reported in Punjab (Ahmad et al., 2005; Durrani et al., 2010). These outbreaks were confirmed through clinical observations and immunoglobulin findings. Genetic characterization of PPRV is very important to understand the epidemiology of PPR outbreaks.

The amplified N gene sequences from both outbreaks (sheep and goats) were found identical. Phylogenetic analysis of N gene demonstrated that PPRV isolates founded in district Gujranwala are clustered in lineage IV which is in agreement of previously reported finding by Munir et al., 2012b in Punjab. An overall tree topology (Fig. 1) revealed that PPRV strains circulating in district Gujranwala resembles to the PPRV strains of Tajikistan (Fig. 1) as reported earlier when PPRV isolates from Punjab coincide with Tajikistan isolates (Munir et al., 2012b). It is fairly promising that PPRV might have come from Tajikistan as they are geographically in close proximity to Pakistan. There is only 36 km distance between Pakistan and Tajikistan through Wakhan border. Pakistan's province Gilgit Baltistan shares its border with the Tajikistan and other countries in the Pamir region where the sheep and goat grazing is very common on the mountains of Pamir. It is also notable that live sheep and goats brought to the whole country from that region to the Punjab during the religious ceremonies like Eid-ul-Adha for selling. This practice can be a source of spreading PPRV from the Pamir region to the whole Pakistan. Therefore it is very important to screen all provinces of Pakistan for PPRV strains for effective control of this economically important disease. Genetic characterization of PPRV is quite compulsory to scrutinize the epidemiological connection and evolutionary relationship between different geographical locations and their exodus. The genetic characterization of local PPRV can help in the early diagnosis and treatment of the disease and can also help in production of local vaccine.

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